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Programmed death-ligand 1 (PD-L1) expression in different sarcoma subtypes

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ABSTRACT

Aims: Sarcomas are rare and heterogeneous mesenchymal malignancies with diverse histological and molecular characteristics. Programmed death-ligand 1 (PD-L1) plays a key role in tumor immune evasion and may serve as a predictive biomarker for immune checkpoint inhibitor therapy. This study aimed to characterize PD-L1 expression across different sarcoma subtypes.

Methods: A total of 54 sarcoma cases diagnosed between January 2020 and September 2025 at the Department of Medical Pathology, Karadeniz Technical University, were retrospectively analyzed. Subtypes included undifferentiated pleomorphic sarcoma (UPS, n=10), liposarcoma (well-differentiated, dedifferentiated, myxoid; n=17), osteosarcoma (n=7), leiomyosarcoma (n=7), myxofibrosarcoma (n=8), and fibromyxoid sarcoma (n=5). PD-L1 expression was assessed immunohistochemically using the validated monoclonal antibody clone 22C3 and quantified by the combined positive score (CPS). PD-L1 positivity was defined as CPS ≥1%.

Results: Overall, 18 of 54 cases (33.3%) exhibited PD-L1 positivity. Expression varied across sarcoma subtypes, with relatively higher positivity rates observed in myxofibrosarcoma (4/8, 50%), leiomyosarcoma (3/7, 42.8%), osteosarcoma (3/7, 42.8%), and UPS (4/10, 25%). Lower PD-L1 expression was observed in fibromyxoid sarcoma (1/5, 20%) and liposarcoma (3/17, 17.6%). Among the liposarcoma subtypes, PD-L1 positivity was detected in one well-differentiated, one dedifferentiated, and one myxoid case.

Conclusion: PD-L1 expression in sarcomas is heterogeneous and subtype-dependent. High-grade pleomorphic tumors, particularly myxofibrosarcomas, leiomyosarcomas, and osteosarcomas, appeared to demonstrate relatively more frequent PD-L1 positivity in this cohort. These findings provide insights into the immunobiology of sarcomas and support the potential rationale for future studies evaluating PD-1/PD-L1-targeted immunotherapies in PD-L1-expressing sarcoma subtypes.

Keywords: Sarcoma, PD-L1, immunohistochemistry, combined positive score, immune checkpoint

INTRODUCTION

Sarcomas are rare and heterogeneous malignant neoplasms of mesenchymal origin, comprising over one hundred histological subtypes with diverse morphological, genetic, and clinical features. This heterogeneity is reflected not only in their histopathology but also in molecular alterations, tumor microenvironment composition, biological behavior, and response to therapy. Genomic complexity, lineage-specific translocations, tumor grade, and interactions between tumor and stromal-immune components collectively influence tumor aggressiveness, metastatic potential, and treatment outcomes. Consequently, identifying reliable biomarkers that predict prognosis and therapeutic response remains a major challenge in sarcoma research.[1,2]

Recently, increasing attention has focused on the tumor immune microenvironment. Interactions between tumor cells and infiltrating immune cells, including lymphocytes and macrophages, play a key role in tumor progression and immune evasion. Among immune regulatory pathways, immune checkpoint molecules-particularly programmed death-ligand 1 (PD-L1)-have emerged as critical mediators of tumor-induced immunosuppression. PD-L1 is expressed by tumor cells as well as by immune and stromal cells, and through its interaction with the PD-1 receptor on T lymphocytes, it inhibits T-cell activity, thereby facilitating immune escape. In several solid tumors, PD-L1 expression has been associated with response to immune checkpoint inhibitors and has been investigated as both a predictive and prognostic biomarker. This is particularly relevant in sarcomas, where conventional treatments-surgery, radiotherapy, and chemotherapy-often provide limited benefit in advanced, metastatic, or recurrent disease.[3,4]

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Emerging clinical and retrospective data suggest that immune checkpoint inhibitors may be effective in selected sarcoma subtypes, especially in high-grade or immune-infiltrated tumors. However, PD-L1 expression is highly variable across sarcoma subtypes. Previous studies have reported PD-L1 positivity ranging from 1.4% to over 59%, with higher expression generally observed in high-grade pleomorphic sarcomas and lower or absent expression in subtypes such as synovial sarcoma and Ewing sarcoma. These findings highlight the importance of subtype-specific evaluation of PD-L1 and suggest its potential functional and therapeutic relevance.[5,6]

Clinical trials investigating immune checkpoint inhibitors have provided evidence that a subset of sarcoma patients may derive benefit from immunotherapy. In the multicenter phase II SARC028 trial, pembrolizumab demonstrated objective responses in selected sarcoma subtypes, particularly undifferentiated pleomorphic sarcoma (UPS) and dedifferentiated liposarcoma, highlighting the potential role of immune checkpoint blockade in specific histological groups.[7] Subsequently, the Alliance A091401 study evaluated nivolumab alone or in combination with ipilimumab in patients with metastatic sarcoma and reported improved response rates with combination therapy, suggesting that dual immune checkpoint inhibition may enhance antitumor activity in selected patients.[8]

Additional studies investigating pembrolizumab and nivolumab-based regimens have further supported the concept that sarcomas are not uniformly resistant to immunotherapy. Although overall response rates remain modest and vary considerably among histological subtypes, durable responses have been observed in a subset of patients, particularly in tumors characterized by a more inflamed tumor microenvironment.[9,10] These findings underscore the need for reliable predictive biomarkers and have increased interest in evaluating PD-L1 expression as a potential indicator of immune responsiveness in sarcomas. Nevertheless, the relationship between PD-L1 expression and clinical benefit from immune checkpoint inhibitors remains incompletely understood and warrants further investigation.

In this study, we aimed to evaluate PD-L1 expression across multiple sarcoma subtypes, including undifferentiated pleomorphic sarcoma, liposarcoma, leiomyosarcoma, osteosarcoma, myxofibrosarcoma, and fibromyxoid sarcoma. The study provides a descriptive analysis of PD-L1 distribution across these histological subtypes, offering insights into the heterogeneity of immune checkpoint expression in sarcomas and laying the groundwork for future studies assessing their clinical significance.

METHODS

Ethics

This retrospective study was conducted in accordance with the ethical principles of the Declaration of Helsinki and was approved by the Karadeniz Technical University Rectorate Faculty of Medicine Scientific Researches Ethics Committee (Date: 26.01.2026, Decision No: 7). Authorization for the use of archived pathology materials was obtained from the Hospital Directorate. Owing to the retrospective design, informed consent was waived. All patient data were anonymized prior to analysis, and no identifiable information was included in the study.

Case Selection

This retrospective study will include a total of 54 sarcoma cases diagnosed between January 2020 and September 2025, retrieved from the archives of the Department of Medical Pathology, Karadeniz Technical University Faculty of Medicine. The sample size was determined based on the availability of suitable paraffin-embedded tissue blocks in our institutional archive. The cases are distributed across different sarcoma subtypes as follows: 10 cases of UPS, 17 cases of liposarcoma, 7 cases of osteosarcoma, 7 cases of leiomyosarcoma, 8 cases of myxofibrosarcoma, and 5 cases of fibromyxoid sarcoma. Among the liposarcoma cases, 3 were classified as dedifferentiated liposarcoma, 4 as well-differentiated liposarcoma, and 10 as myxoid liposarcoma.

PD-L1 Immunohistochemical Assessment

Paraffin-embedded tissue blocks were sectioned at a thickness of 4 μm for immunohistochemical evaluation. PD-L1 expression was assessed using a clinically validated monoclonal antibody clone (22C3), which has demonstrated reproducibility and specificity in routine pathological practice. Immunohistochemical staining was performed following the manufacturer's protocol, and both tumor cells and tumor-infiltrating immune cells within the microenvironment were evaluated. PD-L1 positivity was defined as partial or complete membranous staining of tumor cells and/or membranous staining of tumor-associated immune cells, in accordance with the CPS scoring methodology. Cytoplasmic staining alone was not accepted as positive. Non-specific background staining and staining of necrotic areas were excluded from evaluation.

PD-L1 expression was quantified using the combined positive score (CPS), calculated as the number of PD-L1-positive tumor cells plus PD-L1-positive immune cells, divided by the total number of viable tumor cells, multiplied by 100:

$$\text{CPS} = \frac{\text{PD-L1-positive tumor cells+PD-L1-positive immune cells}}{\text{total viable tumor cells}} \times 100$$

The CPS ≥1% threshold has been widely used in clinical trials and immunohistochemical studies as a biologically and clinically relevant cut-off for defining PD-L1 expression, particularly in studies evaluating eligibility for immune checkpoint inhibitors across multiple tumor types. For instance, CPS ≥1 has been adopted as the standard positivity threshold in several pivotal studies using the PD-L1 IHC 22C3 pharmDx assay, including KEYNOTE program-based trials, where it has been used for patient stratification and as an exploratory or companion diagnostic cut-off in various solid tumors.[11]

In the present study, and in accordance with previously published literature and established approaches in PD-L1 biomarker research, CPS scores were categorized into two

groups as PD-L1 positive (CPS ≥1%) and PD-L1 negative (CPS <1%). The CPS ≥1 threshold was selected because it is widely accepted as a biologically relevant cut-off indicating detectable PD-L1 expression and has been consistently used in exploratory studies evaluating its potential predictive value across different tumor types, including sarcomas.[12]

All slides were independently evaluated by two board-certified pathologists in a blinded manner. In cases of discordant interpretation, a consensus review was conducted to reach a final decision.

To enhance reproducibility and minimize interobserver variability, staining quality, adequacy of tissue, and scoring criteria were systematically reviewed. This standardized approach allows for reliable assessment of PD-L1 expression across multiple sarcoma subtypes and facilitates comparison with clinicopathological parameters.

Statistical Analysis

This study is retrospective and descriptive in nature. The sample size was not determined based on a prospective power calculation, but rather on the availability of sarcoma cases with technically suitable paraffin-embedded tissue blocks for immunohistochemical analysis in the archives of the Department of Medical Pathology, Karadeniz Technical University Faculty of Medicine. Given the rarity of sarcomas and their highly heterogeneous nature with multiple histological subtypes, achieving a large and evenly distributed cohort in a single-center retrospective study is methodologically and practically challenging. Therefore, the number of cases included was defined by the accessible and evaluable cases available within our institutional archive.

The distribution of cases among sarcoma subtypes was guided by archival availability and tissue adequacy, and numerical equality between subgroups was not a primary objective. The main goal of the study is to describe the distribution of PD-L1 expression across different sarcoma subtypes. Consequently, all statistical analyses were planned as descriptive and hypothesis-generating in nature, aiming to contribute valuable data to the limited existing literature on PD-L1 expression in sarcomas.

Statistical analyses were performed using IBM SPSS Statistics software (version 27.0; IBM Corp., Armonk, NY, USA). Categorical variables were presented as counts and percentages. Comparative analyses of PD-L1 expression across sarcoma subtypes were conducted descriptively, without inferential hypothesis testing designed for definitive conclusions.

This approach allows for an exploratory assessment of PD-L1 expression patterns and their potential clinical relevance while acknowledging the limitations inherent to retrospective single-center studies of rare and heterogeneous tumors. Because of the limited sample size and unequal distribution of cases among sarcoma subtypes, no inferential statistical analyses were performed to compare PD-L1 expression between groups. Therefore, the observed differences in PD-L1 positivity rates should be interpreted as descriptive observations rather than statistically validated associations.

RESULTS

The cases were distributed across different sarcoma subtypes as follows: 10 cases of UPS, 17 cases of liposarcoma, 7 cases of osteosarcoma, 7 cases of leiomyosarcoma, 8 cases of myxofibrosarcoma, and 5 cases of fibromyxoid sarcoma. Among the liposarcoma cases, 3 were classified as dedifferentiated, 4 as well-differentiated, and 10 as myxoid liposarcoma.

PD-L1 expression demonstrated notable variability across the different sarcoma subtypes. Among the UPS cases, 4 of 10 (25%) exhibited PD-L1 positivity. In leiomyosarcoma, PD-L1 was expressed in 3 of 7 cases (42.8%). Within the liposarcoma cohort, 3 of 17 cases (17.6%) were PD-L1 positive, including one of 4 well-differentiated, one of 3 dedifferentiated, and one of 10 myxoid liposarcomas. Osteosarcoma showed PD-L1 positivity in 3 of 7 cases (42.8%), while 4 of 8 myxofibrosarcoma cases (50%) were positive. Finally, PD-L1 expression was observed in 1 of 5 fibromyxoid sarcoma cases (20%). Representative examples of PD-L1 expression in various sarcoma subtypes are shown in **Figure**, illustrating the heterogeneity of PD-L1 positivity across UPS, dedifferentiated liposarcoma, leiomyosarcoma, and fibromyxoid sarcoma.

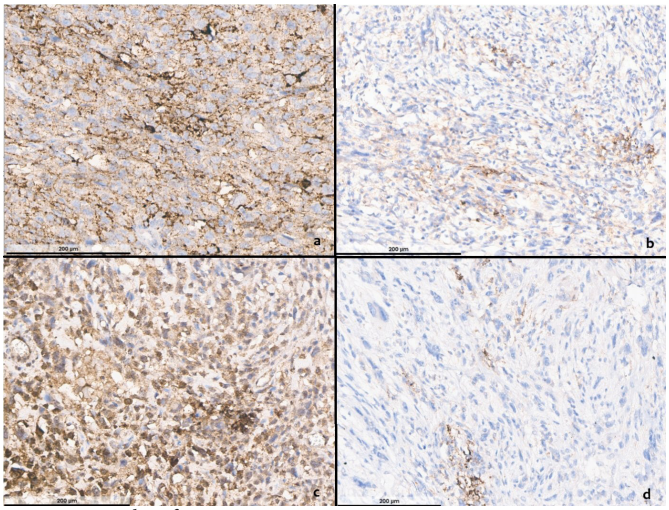

Figure. Examples of PD-L1 expression in various sarcomas

- UPS
- Dedifferentiated liposarcoma
- Leiomyosarcoma
- Fibromyxoid sarcoma

*PD-L1 was assessed using the CPS, with both tumor and immune cells considered. Magnification: ×200. PD-L1: Programmed death-ligand 1, UPS: Undifferentiated pleomorphic sarcoma, CPS: Combined positive score

Overall, 18 of the 54 cases (33.3%) were PD-L1 positive, demonstrating that approximately one-third of the sarcoma cases in this cohort expressed PD-L1. These findings indicate that PD-L1 expression varies among sarcoma subtypes, with the highest positivity observed in myxofibrosarcoma (50%), followed by leiomyosarcoma (42.8%) and osteosarcoma (42.8%), intermediate expression in undifferentiated pleomorphic sarcoma (25%), and lower expression in fibromyxoid sarcoma (20%) and liposarcoma (17.6%). PD-L1 expression varied across the different sarcoma subtypes included in this study, as summarized in **Table**.

Table. PD-L1 expression among various sarcomas

Sarcoma subtype	Total cases (n)	PD-L1 positive cases (n)	PD-L1 positive (%)
UPS	10	4	25%
Leiomyosarcoma	7	3	42.8%
Liposarcoma (total)	17	3	17.6%
Well-differentiated	4	1	25%
Dedifferentiated	3	1	33.3%
Myxoid	10	1	10%
Osteosarcoma	7	3	42.8%
Myxofibrosarcoma	8	4	50%
Fibromyxoid sarcoma	5	1	20%

PD-L1: Programmed death-ligand 1, UPS: Undifferentiated pleomorphic sarcoma

DISCUSSION

In this study, we investigated PD-L1 expression across 54 sarcoma cases spanning multiple histological subtypes. Overall, 33.3% of cases demonstrated PD-L1 positivity, highlighting the heterogeneous nature of immune checkpoint expression in sarcomas. Notably, high-grade and pleomorphic sarcomas, including myxofibrosarcoma (50%), leiomyosarcoma (42.8%), osteosarcoma (42.8%), and UPS (25%), showed relatively higher PD-L1 positivity rates within this cohort. Fibromyxoid sarcoma (20%) and liposarcoma (17.6%) demonstrated the lowest expression levels. These findings underscore the subtype-specific variability in PD-L1 expression and are consistent with previous studies suggesting that high-grade, pleomorphic, and aggressive histologies are more likely to express PD-L1.[5,6]

The observed heterogeneity aligns with prior literature. Large sarcoma series have reported variable PD-L1 expression, with prevalence ranging from 6% to over 60% depending on the cohort and methodology. For instance, Kim et al.[13] reported PD-L1 positivity in 43-65% of sarcomas in Korean series, with higher expression in undifferentiated pleomorphic sarcomas and dedifferentiated liposarcomas. The relatively high expression rates in these studies may reflect differences in antibody clones, staining protocols, cut-off thresholds, and geographic or racial variations.[13,14] Conversely, Western series have generally observed lower PD-L1 prevalence, with studies reporting 6-29% positivity, which is comparable to our findings.[15-17] Taken together, these results emphasize the importance of methodological consistency and the need to consider population-specific factors in PD-L1 research.

Subtype-specific analysis in our cohort further clarifies patterns of PD-L1 expression. Within liposarcomas, PD-L1 positivity was low across well-differentiated, dedifferentiated, and myxoid subtypes, reflecting their lower immunogenicity and limited immune checkpoint activation. In contrast, myxofibrosarcoma, leiomyosarcoma, and osteosarcoma-tumors characterized by higher histologic grade and pleomorphism-exhibited substantially higher PD-L1 expression. This pattern mirrors findings from previous immunohistochemical studies, which suggest that aggressive histology, high mitotic activity, and tumor necrosis correlate with increased PD-L1 expression.[5,6,15] These observations also indicate that PD-L1 expression is not uniformly distributed across sarcomas, and that subtype-specific assessment is essential for accurate biomarker evaluation.

The clinical implications of these findings are significant. High PD-L1 expression in select sarcoma subtypes may identify patients who could benefit from immune checkpoint inhibitor therapy, particularly in high-grade or metastatic disease where conventional treatments such as surgery, radiotherapy, and chemotherapy have limited efficacy. Notably, although low-grade sarcomas generally exhibited low PD-L1 expression, individual cases demonstrating positivity-such as certain myxoid liposarcomas-might be candidates for targeted immunotherapy, potentially preventing progression in metastatic or unresectable disease.[16-18] Moreover, evaluating both tumor cells and tumor-infiltrating immune cells is crucial, as PD-L1 expression in the tumor microenvironment may influence response to PD-1/PD-L1 blockade, even in cases with modest tumor cell positivity.[13,15]

Our study also reinforces the methodological importance of standardized PD-L1 assessment. All cases were independently reviewed by two pathologists, with consensus reached in cases of discordance, and the CPS was utilized to integrate tumor and immune cell expression. This approach enhances reproducibility and aligns with current best practices in immunohistochemical evaluation.[5,6] However, variations in antibody clones, retrieval techniques, and scoring cut-offs remain important considerations when comparing results across studies.[14,19]

Limitations

Several limitations of this study should be acknowledged. First, the sample size was relatively small and subgroup numbers were limited, reflecting the rarity and heterogeneity of sarcomas. Second, the study was retrospective and conducted at a single institution. Third, detailed clinicopathological outcome data, including treatment response, recurrence status, progression-free survival, and overall survival, were not available for all patients. Therefore, correlations between PD-L1 expression and clinical outcomes could not be evaluated, and the clinical significance of the observed PD-L1 expression patterns remains to be determined in future studies.

CONCLUSION

As a result, our findings demonstrate that PD-L1 expression in sarcomas is highly heterogeneous and appears to vary according to histologic subtype and tumor grade. Relatively higher PD-L1 positivity was observed in high-grade, pleomorphic sarcomas-including myxofibrosarcoma, leiomyosarcoma, undifferentiated pleomorphic sarcoma, and osteosarcoma-whereas lower frequencies of PD-L1 expression were noted in low-grade liposarcomas and fibromyxoid sarcomas. These observations suggest potential differences in PD-L1 expression patterns across sarcoma subtypes and may provide a basis for further investigation in the context of immunotherapy. However, given the descriptive and retrospective nature of this study, these findings should be interpreted as preliminary and hypothesis-generating. Future studies with larger multicenter cohorts, standardized

immunohistochemical protocols, and integrated assessment of tumor-infiltrating immune cells, along with comprehensive clinical outcome data, are warranted to better clarify the prognostic and predictive significance of PD-L1 expression in sarcomas.

ETHICAL DECLARATIONS

Ethics Committee Approval

This study was approved by the Karadeniz Technical University Rectorate Faculty of Medicine Scientific Researches Ethics Committee (Date: 26.01.2026, Decision No: 7).

Informed Consent

This retrospective study used pre-existing anonymized patient data. No additional intervention was performed, and there was no direct patient contact. The study was approved by the Ethics Committee, and the requirement for written informed consent was waived by the ethics committee.

Peer Review Process

This manuscript was subject to external peer review.

Conflict of Interest

The authors declare no conflicts of interest related to this study.

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Author Contributions

Concept: GT, İS; Design: GT; Control: GT, İS; Resources: GT; Materials: EK, MSA; Data Collection and/or Processing: GT, EK, MSA; Analysis and/or Interpretation: GT; Literature Review: GT; Writing the Article: GT; Critical Review: GT, EK, MSA, İS.

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